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Neurobiology

Amyloid precursor protein in the cerebral cortex is rapidly and persistently induced by loss of subcortical innervation

(nucleus basalis of Meynert/rat)

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ABSTRACT **Lesions of the cholinergic nucleus basalis of Meynert elevate the *ex vivo* synthesis of β amyloid precursor protein (β-APP) in the cerebral cortex, a major projection region. We have found that this elevation is reflected by increased levels of β-APP mRNA. The induction is rapid (occurring 60 min after placement of the lesion) and persistent (remaining for at least 45 days after lesioning). Two other subcortical lesions, which result in reductions of cortical adrenergic and serotonergic innervation, similarly induced cortical β-APP. The β-APP induction is reversible and does not require loss of the subcortical neurons. Infusion of lidocaine, a calcium antagonist that disrupts neurotransmitter release, into the nucleus basalis of Meynert leads to the temporary reduction of released acetylcholine in the cortex. In this model, β-APP mRNA levels are elevated shortly after the infusion of lidocaine (90 min) but return to preinfusion levels 7 days after the lidocaine treatment. However, metabolic stresses of the brain, including chronic physostigmine, glucocorticoid, and diabetogenic treatments, fail to induce the β-APP response. These results suggest that the induction of β-APP is a specific response to the loss of functional innervation in the cortex. Importantly, these studies show that cortical β-APP is induced by lesions that mimic the neurochemical deficits most frequently observed in Alzheimer disease.**

Among the most prominent features of Alzheimer disease (AD) are profound deficits in cortical cholinergic, noradrenergic, and serotonergic neurotransmitters and the association of amyloid peptide with senile plaques. The reductions of the cortical neurochemical markers for these neurotransmitters appear to be due to the loss of hypofunction of the subcortical neurons responsible for the cortical innervation (1). Amyloid peptide derives from a larger β amyloid precursor protein (β-APP) by an as yet uncharacterized mechanism (2). This observation has led to the proposal that production of the amyloid peptide may be due to selective overexpression of β-APP, particularly in those cortical areas that exhibit an abundance of senile plaques. The relationship between neurotransmitter deficits and β-APP expression has not been elucidated and a unitary paradigm for studying these apparently diverse neuropathological features has not emerged.

We have begun to investigate the relationship between β-APP gene expression and neurotransmitter deficits by determining the effect of neurotransmitter depletion on *ex vivo* β-APP synthesis and β-APP mRNA levels in the cerebral cortex of the rat. Previously, we have shown that lesions of the nucleus basalis of Meynert (nbM) result in increased *ex vivo* synthesis of cortical β-APP 7 days after placement of the lesion (3). The elevated synthesis exhibited specificity to β-APP in that numerous other proteins, including glial fibrillary acidic protein, were not affected.

We have now characterized this lesion-induced β-APP expression more completely and report that (*i*) the elevated *ex vivo* synthesis is due to increased levels of β-APP mRNA; (*ii*) the β-APP response to the lesion is rapid (exhibited within 60 min of lesioning) and persistent (remaining as long as 45 days post-lesion); (*iii*) the induction is reversible, requiring the attenuation of neurotransmitter release but not the loss of the subcortical neurons; (*iv*) subcortical lesions of the cortically projecting noradrenergic and serotonergic systems similarly induce cortical β-APP expression; and, finally, (*v*) other general perturbations of central nervous system function fail to induce the elevated β-APP synthesis response. These results suggest a cause and effect relationship between lesion-induced neurotransmitter deficits and β-APP induction, providing a possible linkage between subcortical neurotransmitter system deficits and amyloid deposition in the AD brain.

METHODS

Placement of Lesions. Adult (≈8 weeks old) male Sprague–Dawley rats (≈225–250 g) purchased from Charles River Breeding Laboratories were subcortically lesioned at the following sites: (*i*) unilateral lesions of the nbM with *N*-methyl-D-aspartate (NMDA, 50 nM) as the excitotoxin as described (3); (*ii*) unilateral lesions of the ascending noradrenergic bundle (ANB) with 6-hydroxydopamine (2 μl of a 4 μg/ml solution) as described (4); (*iii*) dorsal raphe nucleus (DRN) lesions with 5,7-dihydroxytryptamine (50 mM) as described (5). Controls for the nbM and ANB lesions were the contralateral cortices, whereas controls for the DRN lesions were sham-operated animals. This latter sham-operated group also served as controls for any nonspecific contralateral effects produced by the unilateral nbM and ANB lesions.

Transient Inhibition of Cortical Acetylcholine (ACh) Release. A total of 12 male Long Evans hooded rats (300–325 g) was used in this experiment. Each rat was anesthetized with chloral hydrate (400 mg/kg, i.p.) and was positioned in a Kopf stereotaxic apparatus. A 28-gauge cannula was lowered into the region of the nbM (Bregma −0.5, ±3.0, −7.7). One-half of the rats were randomly assigned to group I and also received a 2-mm microdialysis probe (CMA/12; Bioanalytical Systems, West Lafayette, IN) that was stereotaxically directed at the frontal cortex at coordinates Bregma

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Abbreviations: β-APP, β amyloid precursor protein; nbM, nucleus basalis of Meynert; AD, Alzheimer disease; ACh, acetylcholine; Ch, choline; ChAT, Ch acetyltransferase; DRN, dorsal raphe nucleus; ANB, ascending noradrenergic bundle; NMDA, *N*-methyl-D-aspartate.

+3.7, midline +1.8, skull −3.5. The remaining animals were assigned to group II and did not receive microdialysis probes at this time. Approximately 80 min after the introduction of the nbM-directed cannula, a solution of 20% lidocaine in saline was slowly infused into the nbM of all rats at a rate of 0.5 μl/min for 4 min using a microsyringe pump (CMA200). Lidocaine infusions were repeated twice more at 20-min intervals. The rats in group I were sacrificed 90 min after the first infusion of lidocaine and their brains were rapidly removed and frozen on dry ice. The rats assigned to group II were kept anesthetized for an equivalent length of time, were allowed to recover from anesthesia, and were returned to their home cages. One week later the rats in group II were reanesthetized and received microdialysis probes directed at the frontal cortex as had been done with the rats assigned to group I. The release of ACh in the frontal cortex was monitored for 90 min (equal to the time that animals in group I were anesthetized), followed by sacrifice, removal, and freezing of the brain. The dialysate consisted of a Ringers solution containing (nM) 147 Na^+, 3.0 K^+, 1.0 Mg^{2+}, and 1.2 Ca^{2+} and 1 mM neostigmine methyl sulfate. The flow rate was 1.1 μl/min, with fractions collected every 10 min.

Dialysis samples were analyzed by HPLC using a Bioanalytical Systems 460 electrochemical detector and a BSA PM60 pump. A BSA CMA200 autosampler was used to inject 10 μl of dialysis sample. A two-column microbore enzyme reactor kit from BSA was used to separate ACh and choline (Ch). The first column separated the quaternary amines; the second enzyme column converted the Ch to peroxide via Ch oxidase. ACh was hydrolyzed by cholinesterase and the Ch generated was then oxidized. The peroxide was detected by a platinum electrode set at a potential of 500 mV versus an Ag/AgCl reference electrode. The mobile phase consisted of a 50 mM sodium phosphate buffer at pH 8.5. At a flow rate of 100 μl/min, the retention times were 7 min for ACh and 8.9 min for Ch. A detection limit of 100 fmol was attained at twice background current. Results were calculated from peak heights recorded from a dual-pen recorder set at 1.0 V full scale for ACh and 1.0 mV full scale for Ch.

Analysis of mRNA. β-APP mRNA was assayed after isolation of total RNA (6). Equal aliquots of RNA (10 μg) were separated on 1% agarose gels and blotted onto nitrocellulose filter paper. The filters were hybridized [20% formamide, 5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 5× Denhardt's solution, 0.1% SDS overnight at 50°C] with a random primer labeled β-APP cDNA probe (2×10^6 cpm of ^{32}P per ml; 100 ng/ml; generously provided by N. K. Robakis, Mount Sinai School of Medicine) that recognizes all β-APP splice variants. The filters were then washed (twice for 30 min each at 50°C in 0.1× SSC/0.1% SDS) and exposed to film as described (6).

Polysome Assay and Densitometry. *Ex vivo* synthesis of β-APP was assayed exactly as described (3). Briefly, polysomes were isolated from cortical samples, normalized for A_{260} content, and translated in a rabbit reticulocyte lysate system containing [^{35}S]methionine. The control/lesion pairs were normalized for total ^{35}S-labeled protein (as determined by trichloroacetic acid precipitation) and immunoprecipitated with antibodies to the C-terminal region of β-APP (6) (which were provided by N. K. Robakis). The immunoprecipitates were visualized by separation with SDS/polyacrylamide gel electrophoresis and fluorography. The fluorograms were quantitated by laser-directed densitometry (LKB Ultroscan).

The experiments reported herein were conducted according to the principles set forth in ref. 7.

RESULTS

Effects of nbM (Cholinergic) Lesions on β-APP mRNA. One week following the lesion of the nbM, mRNA for β-APP was determined by Northern analysis using a cDNA probe that recognizes all β-APP mRNA isotypes (6). The amount of β-APP mRNA was significantly elevated in the RNA samples made from lesioned cortex compared to its control cortex (Fig. 1). The single message present in the samples reflects the predominance of the mRNA encoding the 695-residue form of β-APP (β-APP_{695}) in rat brain (6). No significant changes were observed when similar samples were probed with actin cDNA. Statistical analysis of the results for the ratio of the densitometric values for β-APP/actin revealed highly significant differences between lesioned and control cortices [$t(df, 4) = 4.7$, $P < 0.005$]. The mean and SEMs for control and lesioned cortices were 0.48 ± 0.06 and 1.05 ± 0.13 densitometric units, respectively. This result indicates an overall elevation of β-APP mRNA concomitant with the elevated *ex vivo* synthesis of the protein (see below and ref. 3).

Time Course of Cortical β-APP Induction by nbM Lesions. Rats receiving unilateral nbM lesions were sacrificed at various times after lesioning. The level of β-APP synthesis was then measured as synthesized by polysomes. *Ex vivo* synthesis of β-APP was significantly increased in the lesioned cortex at all time points after 5 min and up to 45 days after neurotoxin infusion (Fig. 2). A lesion by survival time analysis of variance revealed a significant main effect of lesion [$F(1/27) = 83.7$, $P < 0.0001$] and a significant lesion by survival time interaction term [$F(5/27) = 4.85$, $P = 0.003$]. Aliquots of the tissue homogenates were assayed for the cholinergic marker enzyme Ch acetyltransferase (ChAT) by the method of Fonnum (8). The results of this assay (Fig. 3) demonstrate that ChAT activity decreased steadily until day 3 post-lesion, at which time asymptotic levels were reached. Analysis of variance and Newman–Keuls post-hoc tests revealed a significant effect of survival time [$F(5/30) = 39.4$, $P < 0.00001$]. The infusion of NMDA into the nbM led to a significant decrease in cortical ChAT activity at 1 day post-lesion ($P < 0.01$) followed by a further decrement ($P < 0.01$) in ChAT activity by the third day post-lesion. Cortical ChAT activity was not affected by lesions placed 5 min or 1 hr prior to sacrifice ($P > 0.4$). The lesion-induced ChAT deficits persisted unchanged for the 6-week post-lesion period studied. These results demonstrate that the induction of β-APP synthesis by nbM lesions is time dependent and persists for at least 6 weeks post-lesion (Figs. 2 and 3).

Transient Induction of β-APP by Transient Inhibition of ACh Release. The basal release of ACh in the frontal cortex following the infusion of lidocaine into the nbM was compared to ACh release during the 20-min baseline period immediately preceding lidocaine infusion (Fig. 4*A*). The initial infusion of lidocaine resulted in an average decrease of

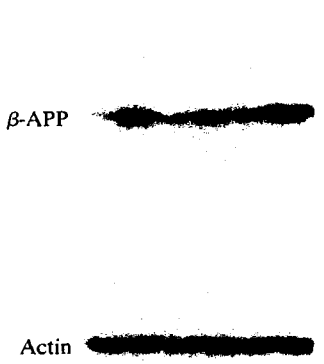

FIG. 1. Levels of β-APP and actin mRNA in control (C) and lesioned (L) cortices. Rat nbM were unilaterally lesioned and total RNA was isolated from contralateral (control) and ipsilateral (lesioned) cortex 7 days later, as described in the text. Total RNA (10 μg) was separated on 1% agarose/formaldehyde gels, blotted onto nitrocellulose filters, and probed with cDNA to either β-APP_{695} (*Upper*) or actin (*Lower*). The filter was then exposed to film for 4 days. Shown are three representative control/lesion pairs (of six that were examined).

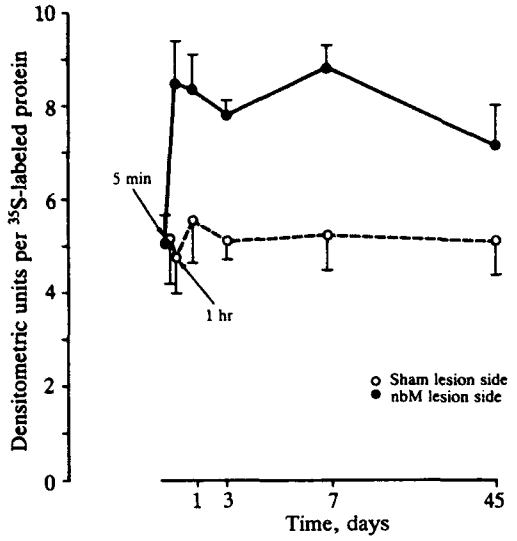

FIG. 2. Synthesis of cortical β-APP various times after lesion of the nbM. Rat brains received unilateral lesions of the nbM. At the indicated times after the placement of the lesion, the animals were sacrificed and β-APP synthesis by the control (○) and lesioned (●) polysomes was assayed as described in the text. Results are presented as densitometric values (arbitrary units) of the immunoprecipitated ^{35}S-labeled β-APP per total ^{35}S-labeled protein from which β-APP was immunoprecipitated. The results are the mean ± SEM of five individual animals. Differences between sham and lesioned sides are significant ($P < 0.002$) except at the 5-min time interval.

ACh release by 25%. Mean ACh release then recovered to near baseline levels during the 60-min period following the first infusion of lidocaine. The time course of maximal ACh release inhibition was variable across different animals. Mean maximal ACh release inhibition was 39.8% of baseline. An analysis of variance comparing the two groups of animals across the post-lidocaine infusion 10-min time epochs revealed a significant effect of lidocaine treatment condition ($F(1/50) = 13.9$, $P < 0.0008$). Post-hoc comparisons (Newman–Keuls) revealed that frontal cortical ACh release was significantly ($P < 0.05$) inhibited during the first three 10-min recording epochs following lidocaine infusion, relative to the same time points in the animals in which ACh release was measured 1 week following lidocaine infusion. Levels of β-APP mRNA in the cortex were assayed in animals sacrificed either shortly after the infusion of lidocaine (90 min) or 7 days after the infusion (Fig. 4*B*). Samples from animals sacrificed shortly after lidocaine infusion exhibited a similar induction of β-APP as observed in the subcortically lesioned rat. Similar to the results shown in Fig. 1, actin mRNA levels were not affected by the lidocaine treatment. In contrast to the results obtained in animals sacrificed shortly after lidocaine infusion, samples obtained from the rats surviving 7 days after lidocaine infusion exhibited no differences between the treated and control cortices. The Northern blots shown in Fig. 4*B* were analyzed by densitometry to quantitate the mRNA levels. Significant differences were observed between the treated cortex and its contralateral side control in the short-term-survival animals ($n = 6$ per group, $P < 0.001$, Newman–Keuls) but not in the 7-day-survival rats ($n = 6$ per group, $P > 0.3$).

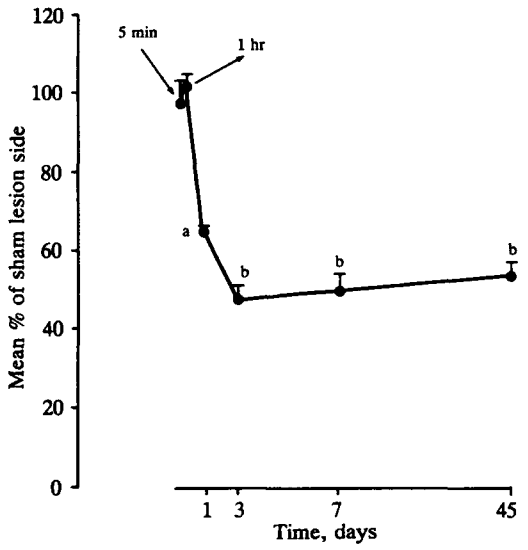

FIG. 3. Depletion of cortical ChAT at various times after lesion of the nbM. Aliquots of tissue homogenates used for determinations of β-APP synthesis were assayed for ChAT activity by the method of Fonnum (8). Results are presented as mean % ChAT activity (± SEM) in the cortex ipsilateral to the lesion relative to the nonlesioned side (a: vs. 5-min, 1-hr, 3-day, 7-day, and 45-day time points, $P < 0.01$; b: vs. 5-min, 1-hr, and 1-day time points, $P < 0.01$).

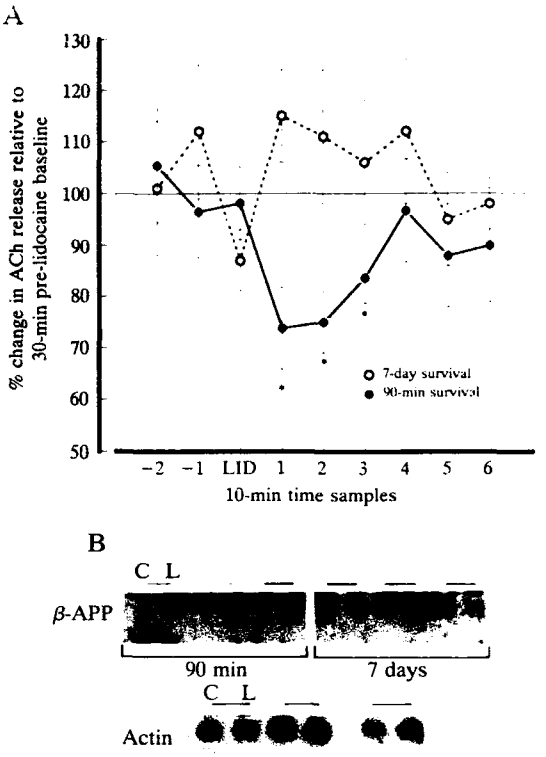

FIG. 4. Effects of infusion of 20% lidocaine into the nbM on cortical levels of released ACh and β-APP mRNA. Animals received infusions of 20% lidocaine into the nbM as described in the text. The contralateral nbM was used as the control. Microdialysis probes were placed into the frontal cortex of some animals ipsilateral to the side of lidocaine (LID) infusion to measure the cortical release of ACh (*A*). Shown are mean percent changes in released cortical ACh during the infusion of lidocaine (●) and at 7 days after the infusion of lidocaine [○; * denotes significant ($P < 0.05$) differences between groups]. To measure β-APP mRNA levels, animals were sacrificed either 90 min or 7 days after lidocaine infusion. Total RNA was isolated from cortices ipsilateral (L) and contralateral (C) to the nbM infused with lidocaine. β-APP mRNA was determined on Northern blots. Shown are three representative pairs (of six assayed) each of the short-term-survival and long-term-survival samples. The three short-term RNA samples assayed for actin mRNA are shown (*B*).

Effects of Forebrain Noradrenergic and Serotonergic Lesions on the *ex Vivo* Synthesis of β-APP. To investigate the

Table 1. Effects of lesions of nbM, ANB, and DRN on cortical neurotransmitter markers and β-APP synthesis

Tissue	Transmitter marker*	%†	β-APP synthesis‡	%†
nbM control	39.1 ± 1.3		3.03 ± 0.42	
nbM lesion	18.1 ± 0.9§	54	6.84 ± 0.53§	225
ANB control	0.25 ± 0.01		3.57 ± 0.39	
ANB lesion	0.13 ± 0.01§	58	6.30 ± 0.52§	180
DRN control	0.12 ± 0.005		2.67 ± 0.25	
DRN lesion	0.05 ± 0.005§	34	5.74 ± 0.62§	224

*For nbM, ChAT (nmol of ACh per hr per mg of protein); for ANB, norepinephrine (μg/g of wet weight); for DRN, serotonin (μg/g of wet weight).
†% of control.
‡Densitometric units per total ^{35}S-labeled protein.
§$P < 0.01$ by Student's t test (two-tailed).

transmitter specificity of the β-APP induction response, we determined the cortical synthesis of β-APP in animals with lesions of the forebrain cholinergic, noradrenergic, and serotonergic systems. Animals were given lesions of the nbM, DRN, or ANB as described in *Materials and Methods*. After a 1-week survival, the cortices were assayed for markers of the three neurotransmitter systems and β-APP synthesis by polysomes. The results of the neurochemical studies (Table 1) indicated that each lesion procedure led to a significant and specific deficit in the cortical levels of the corresponding transmitter markers. As observed for the cholinergic lesion in previous studies (3) and replicated here, cortices from ANB and DR lesioned rats also exhibited the increases of newly synthesized β-APP (Fig. 5). After quantitation of the immunoprecipitates by densitometry and analysis of variance, there was a significant main effect of lesion [$F(1/24) = 68.67$, $P < 0.00001$]. Post-hoc comparisons using Newman–Keuls tests showed significant ($P < 0.01$) differences between each set of lesioned vs. control tissues. There were no significant differences between nonlesioned cortices irrespective of lesion group condition ($P > 0.1$).

Effects of Various Pharmacological and Systemic Treatments of Rats on *ex Vivo* β-APP Synthesis. The induction of cortical β-APP by lesions of cortically projecting cholinergic, noradrenergic, and serotonergic systems raises the question of whether physiologically stressful conditions or perturbations of normal physiological functions in general regulate the synthesis of β-APP. This possibility was investigated in three experiments. (*i*) Different groups of rats were chronically treated with s.c. doses of physostigmine (0.5 mg/kg for 1 week and 0.25 mg/kg for an additional week) or saline ($n = 5$). (*ii*) In collaboration with A. Miller (Mount Sinai School of Medicine) and R. Spencer (The Rockefeller University), rats received either sham operations or were adrenalectomized. The adrenalectomized animals were treated chronically either with saline or with a high dose of the potent type II adrenal-steroid receptor agonist RU 28362 (9) (administered by Alzet mini pumps at 10 μg/hr for 7 days; $n = 5$). (*iii*) In collaboration with S. Hoyer (Heidelberg University, Germany), different groups of rats were treated intracerebroventricularly either with artificial cerebrospinal fluid (aCSF) or with aCSF plus the diabetogenic compound streptozotocin (1.25 μg/g of body weight; $n = 4$) for 42 days, resulting in significant reduction of brain energy metabolism as indicated by a 47% decrease in ATP/ADP ratios (10). The rats in all three studies were sacrificed, cerebral cortices (for the physostigmine and streptozotocin experiments) or cerebelli (for the adrenalectomy and RU 28362 experiment) were dissected, and β-APP synthesis was investigated by the polysome translation methods described in *Materials and Methods*. Analysis of the results (Table 2) revealed that none of the treatments significantly affected the *ex vivo* synthesis of β-APP relative to untreated controls (all $t < 1.1$, all $P > 0.1$).

Table 2. Effects of various stresses on β-APP synthesis

Treatment	n	β-APP synthesis*
Physostigmine		
Control	5	4.27 ± 0.27
Treated	5	4.67 ± 0.55
Glucocorticoid		
Sham	4	1.88 ± 0.64
Control	4	1.45 ± 0.31
Treated	4	1.42 ± 0.25
Streptozotocin		
Control	5	2.58 ± 0.66
Treated	5	3.24 ± 0.91

*Densitometric units per total ^{35}S-labeled protein.

FIG. 5. Effects of forebrain noradrenergic and serotonergic lesions on cortical β-APP synthesis. Animals received lesions of the ANB (*A*) or the DRN (*B*) as described in the text. After 1 week, the animals were sacrificed and β-APP synthesis was assayed on cortical polysomes. Shown are immunoprecipitates from four representative control (C)/lesion (L) pairs (of six) for each lesion.

DISCUSSION

In an initial investigation, we found that lesions of the nbM resulted in increased synthesis of β-APP by purified polysomes in the cortical projection areas 7 days after lesioning (3). Polysomes that are isolated from tissue contain mRNA in the process of synthesizing their corresponding polypeptides *in vivo*. Therefore, the increased synthesis of β-APP by the lesioned cortical polysomes suggests that β-APP synthesis is elevated *in vivo*. However, because the cortical polysomal mRNA is translated *in vitro* in a heterologous assay system, it is possible that β-APP mRNA is in some way preferentially translated and does not accurately reflect the rate of β-APP synthesis *in vivo*. This induction of cortical β-APP has been further characterized to better understand the normal role of β-APP in the intact brain and the potential role of β-APP in subsequent pathological processes.

The increased synthesis of β-APP by cortical polysomes in the lesioned animal was corroborated by the observation of elevated β-APP mRNA in the lesioned samples (Fig. 1). The increased mRNA suggests that the expression of β-APP was due to the greater transcription of the β-APP gene, although our observations could also be explained by enhanced stability of the β-APP message after the lesion. Due to its predominance in the rat brain (6), only the β-APP$_{695}$ splice variant was directly examined in this assay. The equal elevation of each protein isotype as seen in the immunoprecipitates suggests that all splice variants are induced with the lesion.

The time course of the β-APP induction shows that it is rapid and persistent (Fig. 2). The initial induction of β-APP at 1 hr may be obtained by either transcriptional or translational up-regulation, or both. The rapid induction of β-APP post-lesion suggests that the induction of β-APP is not a function of the physical loss of the cortical cholinergic synapse. Since cortical ChAT activity, and presumably the

cholinergic neurons containing it, are lost much more slowly following nbM lesions (11, 12) (Fig. 3: no change in ChAT activity at 60 min post-lesion, vs. maximal β-APP induction at this time point), it is unlikely that the rapid induction of β-APP synthesis by cortical polysomes is due to the physical loss of cortical synapses with the nbM neurons. The immediate and short-term effects of NMDA infusion into the nbM on cortical ACh levels are unclear. However, electrophysiological data suggest that the exposure of neurons to high doses of NMDA results in an almost immediate depolarization of the affected neurons (13). This observation suggests that the release of ACh at the cortical synapse is prevented very soon after the infusion of NMDA. Therefore, the projection areas in the cortex may be responding specifically to the absence of neurotransmitter at postsynaptic cortical sites with the induction of β-APP. To determine whether physical damage to the subcortical neurons was a necessary condition for the induction of β-APP, the nbM projecting to one cortical hemisphere was infused with lidocaine, a calcium antagonist that reversibly disrupted the cortical release of ACh (14). Immediately after lidocaine infusion, cortical release of ACh was reduced (Fig. 4*A*). Concomitant with this decrease in cortical cholinergic neurotransmission, there was an elevation of cortical β-APP mRNA levels (Fig. 4*B*). With time after the infusion of lidocaine, cortical ACh levels returned to normal as did the amount of cortical β-APP mRNAs. These results indicate that (*i*) a diminution in the release of transmitter in the cortex is sufficient to induce cortical β-APP and (*ii*) this induction is reversible with the resumption of subcortical neuronal function. These results support the view that the induction of cortical β-APP is triggered by the loss or diminution of neurotransmitter at the postsynaptic site. The reversibility of the induced β-APP in this paradigm suggests that normalization of cortical neurotransmitter activity can reverse the induction of β-APP.

In addition to the forebrain cholinergic deficits in AD, serotonergic and noradrenergic systems are also affected (15). Results from a variety of experiments in animals, such as those reported here, suggest that lesions of the ANB and the DRN produce severe decrements in cortical noradrenergic and serotonergic markers (4, 5). Such lesions also produce impairments in cognitive function, arousal state, and responsivity to pharmacological agents (4, 5). Lesions of these structures resulted in a similar induction of β-APP as seen with nbM lesions (Fig. 5). These results indicate that cortical β-APP is induced not only by forebrain cholinergic lesions (3) but also by lesions that affect cortical noradrenergic and serotonergic systems. It is important to note, however, that lesion-induced increases in polysomal β-APP synthesis are not likely to be a direct result of neurotoxins or nonspecific damage to the central nervous system. This conclusion is supported by the fact that polysomal synthesis of β-APP is (*i*) induced by lesions produced by different neurotoxins with different mechanisms of action (i.e., cholinergic system, NMDA; noradrenergic system, 6-hydroxydopamine; serotonergic system, 5,7-dihydroxytryptamine); (*ii*) induced by the actual disruption of neurotransmission since sham lesions, including cannula insertion into the lesion site, fail to affect cortical *ex vivo* β-APP synthesis; (*iii*) reversible and dependent upon the attenuation of neurotransmitter release; and (*iv*) not induced in at least one region of the brain, the cerebellum (data not shown), which does not receive a direct cholinergic projection from the nbM. These findings substantiate the relationship between subcortical lesion-induced neurotransmitter deficits and synthesis of β-APP by cortical polysomes. These studies have not established that increased β-APP synthesis is necessarily specific to lesions of only those systems known to be affected in AD. Induction of β-APP may be a physiologic consequence of the general loss of synaptic activity. Lesions of other cortically projecting systems (such as the dopaminergic system) are also likely to induce cortical β-APP.

Despite the induction of cortical β-APP by a variety of lesions, β-APP induction is not a simple response to general stresses. Three different perturbations of cerebral function did not induce β-APP (Table 2). The results of these three negative experiments demonstrate that *ex vivo* β-APP synthesis is not influenced by the increased availability of ACh in the cortex (physostigmine treatment) or by generalized central or peripheral stressors (RU 28362 and streptozotocin). These experiments cannot exclude the influence of these agents and manipulations on β-APP synthesis in other tissues or completely rule out the influence of other stressors on cortical β-APP. These results do suggest, however, that the induction of β-APP is not a central response to generalized stress or physiological disturbance and is not observed, at least within the parameters thus far investigated, under conditions where specific central nervous system pathways have not been directly disrupted.

These results indicate that cortical projection areas respond to the loss of subcortical innervation with a rapid and persistent induction of β-APP. This observation suggests that one function of β-APP is involved with interneuronal communication in the intact brain. The determination of the precise physiological signal for the induction will be important for our understanding of the central nervous system response to injury. That the induction of β-APP is dependent upon the diminution of neurotransmitter activity at the cortical synapse suggests that changes in neurotransmitter activity are among the processes that regulate β-APP and raises the possibility that pharmacological agents that enhance cortical neurotransmitter activity may prevent or diminish the induction of β-APP.

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